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HEXURONIC ACID AS THE ANTISCORBUTIC FACTOR

EXPERIMENTS are being carried out in order to decide whether 'hexuronic acid' is the antiscorbutic factor. So far as is known, the distribution of this acid in plants follows closely the distribution of vitamin C. In the animal body it can also be found in relatively high concentration in the suprarenal cortex. Its chemical properties closely agree with the known properties of the vitamin. It was discovered and isolated several years ago at the Biochemical Laboratory, Cambridge.¹

The hexuronic acid used in the present series was prepared in crystalline form from beef suprarenal glands two years ago at the Chemical Department of the Mayo Clinic.² As is known, 1.5 c.c. of lemon juice is the minimum protective dose for guinea-pigs against scurvy. This quantity of lemon juice contains approximately 0.5 mgm. of hexuronic acid. 1 mgm. of the acid has been given to our test animals daily, since, owing to the long exposure to air, some of our hexuronic acid preparation may have been decomposed.

The general procedure used in studying the antiscorbutic activity of hexuronic acid was that recommended by Sherman and co-workers.³

The test period in the first experiment consisted of 56 days. At the end of that time the guinea-pigs which had been receiving hexuronic acid, as well as the positive controls which received 1 c.c. of lemon juice, were chloroformed. The positive controls showed mild scurvy on autopsy, while the animals receiving hexuronic acid showed no symptoms of scurvy at all. The negative controls, which received the basal diet only, had an average survival of 26 days and had typical symptoms of scurvy. In this experiment, however, only a small number of animals were used, and the animals receiving hexuronic acid, as well as the positive controls, were losing weight continually because the basal diet employed at that time contained no milk powder (it consisted of rolled oats, bran, butter fat, and salt). For this reason we decided to repeat the experiment.

In the test which is in progress at the present time the defects mentioned above have been remedied. A

large number of animals has been used, and skimmed milk powder has been added to the basal diet.

The test was composed of the following groups: (1) Negative controls receiving the basal diet only, 9 animals. (2) Positive controls, receiving 1 c.c. of lemon juice daily, 8 animals. (3) Test animals receiving the basal diet and 1 mgm. of hexuronic acid daily, 10 animals. (4) Controls receiving mixed diet, 10 animals.

The negative controls all died between the time limit of 20-34 days, with an average survival of 26 days, after a continuous and big drop of weight. They all had symptoms of severe scurvy.

At the end of 55 days all the animals receiving hexuronic acid, as well as the positive controls with lemon juice or mixed diet, were living apparently in good health and were gaining weight consistently. At this time three animals which received hexuronic acid and two animals which received lemon juice were chloroformed. Mild symptoms of scurvy were present in the positive controls with lemon juice, but no signs of scurvy in the animals receiving hexuronic acid.

The test will be continued until the ninety-day period is over, and full details will be published later.

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¹ Szent-Györgyi, A., *NATURE*, May 28, 1927; *Biochem. J.*, **22**, 1387; 1928.

² Szent-Györgyi, A., *J. Biol. Chem.*, **90**, 385; 1931.

³ Sherman, H. C., La Mer, H. K., Campbell, H. L., *J. Am. Chem. Soc.*, **44**, 165; 1922.

At the wish and by the courtesy of Prof. A. Szent-Györgyi, I arranged to examine in my laboratory the 'hexuronic acid' which he isolated while working in the Biochemical Laboratory, Cambridge. At the end of 1929 he sent me 10 grams of the substance, which had been prepared in the chemical laboratory of the Mayo Clinic, Rochester, U.S.A. Owing to the value and scarcity of this material, it has been necessary to carry out each experiment with very small quantities, and to establish with much deliberation and care the experimental conditions and controls. This work is still in progress and is being directed to the elucidation of the constitution and the achievement of the syn-

thesis of the substance ; this has involved the study of its chemical properties, and the formation of a crystalline derivative. The preliminary results now communicated show that the hexuronic acid is most probably the 6-carboxylic acid of a keto-hexose, which does not appear to be related either to *d*-fructose or to the ketose corresponding to *d*-galactose. This work has been conducted by my colleague Dr. E. L. Hirst, assisted by Mr. R. J. W. Reynolds, whose report is given in the accompanying note.

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THE 'hexuronic acid' prepared from suprarenal glands by Prof. Szent-Györgyi was a cream-coloured micro-crystalline powder, m.p. 184°-187° (decomp.). On recrystallisation from methyl alcohol-ether the substance was obtained in irregular aggregates of rectangular crystals, which were almost colourless. No change in m.p., analysis, or other properties was observed even after several successive crystallisations. The crystals showed brilliant colours when observed between crossed nicols in a polarising microscope. Before and after recrystallisation the same analytical figures were obtained (Found: C, 41.0; H, 4.7. $C_6H_8O_6$ requires C, 40.9; H, 4.5 per cent). Neither nitrogen nor methoxyl was present. In aqueous solution the rotation $[\alpha]_D^{20} + 23^\circ$ (c. 1.1) increased slowly to $+31^\circ$ (3 days) and then decreased to zero (11 days).

The hexuronic acid reduced Fehling's solution, neutral silver nitrate, and neutral potassium permanganate in the cold. It gave the Molisch test and the orcinol reaction, but failed to show the naphtorescein colour test characteristic of glycuronic acid.

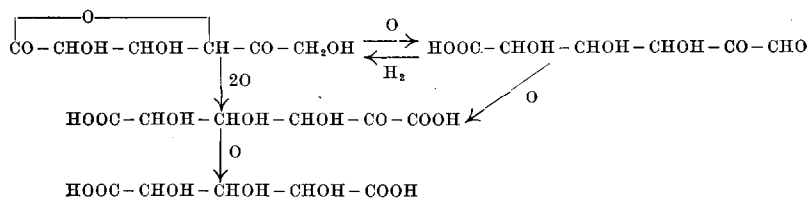
The hexuronic acid was monobasic (40 mgm. of sodium hydroxide neutralised 172 mgm. of substance—calc. for $C_6H_8O_6$, 176).

Oxidation by atmospheric oxygen in slightly alkaline solution, with a trace of copper as catalyst, introduced one carboxyl group in place of a primary alcohol group and the product reduced Fehling's solution. Oxidation to the same stage occurred with remarkable rapidity when the hexuronic acid reacted with neutral, acid, or slightly alkaline potassium permanganate. The reaction, which required two atoms of oxygen per molecule of the substance, was thereafter much less rapid but proceeded regularly in the cold until

one further atom of oxygen per molecule had been absorbed. The product was now non-reducing.

When heated with phenylhydrazine in dilute acetic acid, the hexuronic acid gave intractable, dark-coloured, amorphous products which could not be purified. The action of *p*-bromophenylhydrazine in dilute acetic acid on the barium salt of the hexuronic acid (compare Goldschmiedt and Zerner, *Monatsh.*, **33**, 1217, 1912) gave a dark red micro-crystalline powder which, after recrystallisation from alcohol, had m.p. 230°-235° (decomp.). Analysis showed it to be the *p*-bromophenylosazone of a hexose-uronic acid (Found: C, 40.8; H, 3.5; N, 11.1; Br, 30.3. $C_{18}H_{18}O_5N_4Br_2$ required C, 40.7; H, 3.4; N, 10.6; Br, 30.2 per cent). Control experiments with glycuronic acid and galacturonic acid failed to give the above substance. With glycuronic acid Goldschmiedt and Zerner's barium salt of the *p*-bromophenylosazone of glycuronic acid was obtained, whilst the galacturonic acid gave a yellow powder which appeared to be mainly the barium salt of the corresponding galacturonic acid derivative.

The above reactions, together with Prof. Szent-Györgyi's observation that oxidation by iodine in the cold removes two atoms of hydrogen, which are easily replaced by mild reducing agents, can be understood most readily on the following basis :



Inasmuch as the configuration and ring-structure of the lactone of the hexuronic acid have not yet been established, the structural formulæ are given above in the open chain form, although the sugar-ring is most probably that of a keto-furanose.

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